Supplementary material

In vitro susceptibility of *Leishmania infantum* to Artemisinin derivatives and selected trioxolanes

Sofia Cortes[a,b]#, Andreia Albuquerque[a], Lília I. L. Cabral[c,d], Liliana Lopes[a,d], Lenea Campino[a,d] , Maria L. S. Cristiano[c,d]#

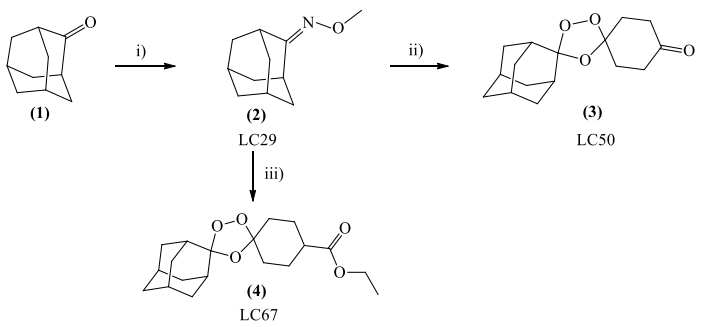
1. Synthesis of compounds tested

1.1 General methods

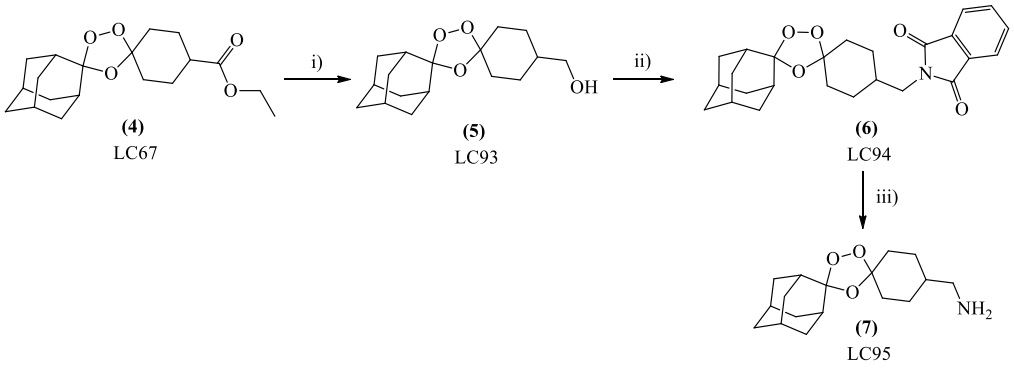
Commercial reagents were used as purchased. When required, solvents were dried following standard procedures[1]. ^{1}H and ^{13}C NMR spectra were recorded on a 400 MHz NMR spectrometer. ^{1}H NMR-chemical shifts are referred to the residual signal of $CDCl_3$ (δH 7.27) and ^{13}C NMR- chemical shifts to the $CDCl_3$ signal (δC 77.0), or using TMS as internal standard. Thin-layer chromatography was carried out on silica gel 60 F254 plates (AL TLC 20x20). Column chromatography was performed on Silica Gel 60 (0.04 – 0.063 mm). IR spectra were recorded on a Tensor 27 FT/IR spectrometer in the 600–3800 cm^{-1} range. Melting points (°C) were obtained on a "SMP3 Melting Point Apparattus and are uncorrected.

1.2 Preparation of trioxolanes

The synthetic approach followed to trioxolanes is depicted in Schemes 1,2. Synthetic procedures for each compound prepared are also provided in this section.

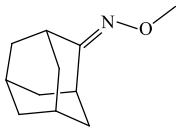


Scheme 1: Synthetic route to trioxolanes LC50 and LC67. Reagents and conditions: i) Pyridine, $MeONH_2$, MeOH; ii) 1,4-Cyclohexane, O_3, DCM/pentane, -78ºC; iii) Ethyl 4-oxocyclohexanecarboxylate, O_3, DCM/Pentane, -78ºC.



Scheme 2: Synthetic route to trioxolane LC95 from LC67. Reagents and conditions: i) $LiBH_4$, Et_2O, $LiBH(Et)_3$, r.t.; ii) Phthalimide, Ph_3P, DIAD, THF, 0ºC; iii) Hydrazine hydrate, chloroform/MeOH, 60ºC.

1.1.1. ***Preparation of O-methyl-2-adamantanone oxime (2)***.



(2)
LC29

To a solution of 2-adamantanone (4.51 g, 30 mmol) in methanol (30 mL) were added pyridine (4.5 mL, 55.6 mmol) and methoxylamine hydrochloride (3.76 g, 45.0 mmol). The reaction mixture was stirred at room temperature for 48 h. The final mixture was concentrated and then diluted with DCM (50 mL) and water (50 mL). The organic layer was separated and the aqueous layer was extracted with DCM (30 mL). The combined organic extracts were washed with aqueous HCl (1 M; 30 mL x2), then with saturated aqueous NaCl (30 mL). The final organic extract was dried over $MgSO_4$, filtered and concentrated under reduced pressure to give *O*-methyl-2-adamantanone oxime (4.77 g, 26.6 mmol, 89%) as a colorless solid (m.p. 69-70ºC). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.78-1.97 (12H, m), 2.53 (1H, s), 3.45 (1H, s), 3.81 (3H, s); MS (MALDI-TOF): m/z 180.02 $[M]^+$.

1.1.2. Synthesis of 1,2,4-trioxolanes

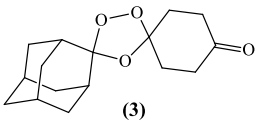
General procedure

Trioxolanes were prepared by coupling O-methyl-2-adamantanone oxime (2) with a cyclohexanone derivative, through ozonolysis.

Ozone, produced with an ozone generator Sander Labor-Ozonizator 301.7 (0.5 L/min O_2, 140 V), was passed through a solution of dichloromethane at –78ºC and flushed into a solution of

O-methyl ketone oxime and a ketone, in pentane/dichloromethane (6:4) at 0ºC. After completion, the solution was flushed with nitrogen for 5 min and concentrated under reduced pressure at room temperature to give a crude material that was purified by column chromatography.

Adamantane-2-spiro-3'-8'-oxo-1',2',4'-trioxaspiro[4,5]decane (3).

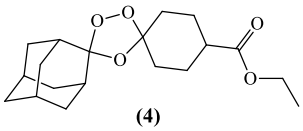


(3)

LC50

A solution of *O*-methyl 2-adamantanone oxime (1.5 g, 8.4 mmol) and 1,4-cyclohexanedione (1.9 g, 11 mmol) in pentane (60 mL) and dichloromethane (40 mL) was treated with ozone (as described in general procedure I). The crude product was purified by column chromatography (silica gel; ethyl acetate/n-hexane 1/9) to give product **3** (1.18 g, 3.52 mmol, 42%) as a colorless solid (m.p. 127-128ºC). ^{1}H-NMR ($CDCl_3$): δ 1.69-2.02 (m, 14H), 2.14 (t, J = 6.9 Hz, 4H), 2.51 (t, J = 7.0 Hz, 4H); ^{13}C NMR (100 MHz, $CDCl_3$): 25.9, 26.31, 31.09, 32.59, 34.25, 35.70, 36.18, 37.35, 106.46, 111.95, 208.90; MS (EI), m/z 278.9 $[M]^+$.

Adamantane-2-spiro-3'-8'-ethoxycarbonyl-1',2',4'-trioxaspiro[4,5]decane (4).

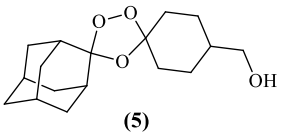


(4)

LC67

A solution of *O*-methyl 2-adamantanone oxime (3.58 g, 20 mmol) and ethyl 4-oxocyclohexanecarboxylate (3.40 g, 20 mmol), in pentane (60 mL) and DCM (40 mL), was treated with ozone, according to the previous procedure. The crude product was purified by column chromatography (silica gel, ethyl acetate/n-hexane 1/9) to afford trioxolane **4** as a colorless oil (3.10 g, 9.2 mmol, 46%). ^{1}H-NMR ($CDCl_3$): δ 1.26 (3H, t, J=6.9Hz), 1.70-1.76 (11H, m), 1.92-2.03 (12H, m), 2.33 (1H, m), 4.15 (2H, dd, J=7.2Hz, J=14.3Hz); MS (MALDI-TOF), m/z 337.34 $[M]^+$.

Adamantane-2-spiro-3'-8'-hydroxymethyl-1',2',4'-trioxaspiro[4,5]decane (5).



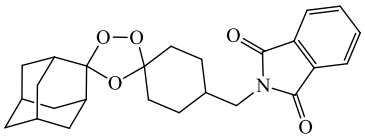
(5)

LC93

A solution of **4** (3.8 g, 11.3 mmol), lithium borohydride (5.7 mL, 11.3 mmol, 2M in THF) and lithium triethylborohydride (1.15 mL, 1.13 mmol, 1M in THF) in ether (15 mL) was

stirred overnight, at rt. The reaction mixture was diluted with ether (5 mL), washed with aqueous NaOH (3M; 2 x 10 mL), brine and water (2 x 10 mL). The organic extract was dried over $MgSO_4$, filtered, and concentrated under reduced pressure to give product **5** (3 g, 10.2 mmol, 90%) as a colorless solid (m.p. 99-101ºC). ^{1}H-NMR ($CDCl_3$): δ 1.25 (2H, m), 1.51-2.08 (21H, m), 3.46 (2H, t, J=4.8Hz); MS (MALDI-TOF), m/z 318.30 $[M+Na]^+$.

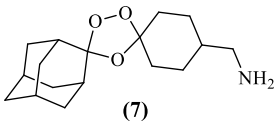
Adamantane-2-spiro-3'-8'-phtalimidomethyl-1',2',4'-trioxaspiro[4,5]decane (6).



(6)
LC94

A solution of **5** (2.8 g, 9.52 mmol) in dry THF (25 mL) was cooled to 0 °C. Ph_3P (3.5 g, 1.33 mmol), phthalimide (1.55 g, 10.5 mmol) and DIAD (2.6 mL, 1.33 mmol) were gradually added. The mixture was stirred at room temperature for 24 hours. The solvent was then evaporated to dryness and the crude product was purified by column chromatography (silica gel, ethyl acetate/n-hexane 1/9) to give product **6** (3.22 g, 7.62 mmol, 80%) as a white powder (.mp. 149-151 ºC). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.30-1.34 (2H, m), 1.51-2.08 (21H, m), 3.55 (2H, d, J=7.0Hz), 7.71 (2H, m), 7.84 (2H, m); MS (MALDI-TOF), m/z 462.19 $[M+K]^+$.

Adamantane-2-spiro-3'-8'-(aminomethyl)-1',2',4'-trioxaspiro[4,5]decane (7).

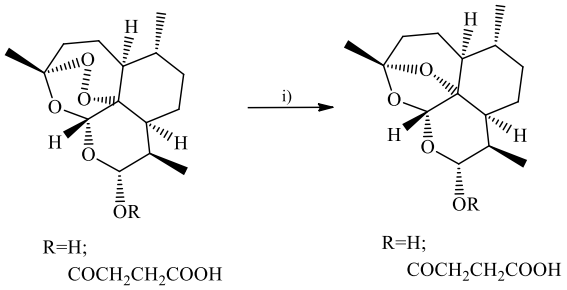


(7)
LC95

A solution of **6** (3.20 g, 7.56 mmol) and hydrazine monohydrate (1.45 g, 45.4 mmol) in chloroform and methanol (7:3, 50 mL total) was heated at 60ºC for 48 h. The reaction mixture was cooled to room temperature and filtered to remove solid by-products. The filtrate was washed with water (50 mL) and brine (50 mL), dried over $MgSO_4$, filtered, and concentrated to give product **7** (1.72 g, 5.87 mmol, 77%) as light yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.14-1.33 (3H, m), 1.68-1.96 (22H, m), 2.54 (2H, d, J=6.4Hz); MS (MALDI-TOF), m/z 293.20 $[M]^+$.

1.2. Preparation of deoxygenated artemisinin derivatives

Deoxygenated artemisinin derivatives(deoxy-DHA and deoxy-artesunate) were prepared from the parent compounds, through reduction, as depicted in Scheme 3, following a methodology described in the literature (Copple, 2012). A detailed description of the reduction procedure is provided in this section.



Scheme 3: Strategy for desoxygenation of DHA and Artesunate. Reagents and conditions: i) Zn powder, glacial acetic acid, 60ºC.

1.2.1. Preparation of deoxy-dihydroartemisinin

Zinc powder (16 g) was added in one portion to DHA (10 g, 35.16 mmol) in glacial acetic acid (120 ml). The mixture was stirred at 60ºC, overnight. The solution was filtered over Celite® and then washed with dichloromethane and ethyl acetate. The crude was purified by column chromatography (silica gel, ethyl acetate/n-hexane 1/9) to afford the product as a colorless crystalline solid (4.17 g, 15.5 mmol, 44 %; m.p. 143-144ºC). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 0.99 (d, J= 6.1 Hz, 3H), 1.43 (s, 3H), 2.40 (td, J= 14.0 Hz and J= 4.2 Hz, 1H), 2.76 (m, 1H), 4.76 (s, 1H), 5.34 (m, 1H), MS (EI), *m/z*, 291.1 $[M+Na]^+$.

1.2.1. Preparation of deoxy-artesunate

Zinc powder (16 g) was added in one portion to artesunate (10 g, 26.01 mmol) in glacial acetic acid (120 ml). The mixture was stirred at 60ºC, overnight. The solution was filtered over Celite® and then washed with dichloromethane and ethyl acetate. The crude was purified by column chromatography (silica gel, ethyl acetate/n-hexane 1/9) to afford the product is a light yellow oil (4.17 g, 11.32 mmol, 44 %). MS (EI), *m/z*: 367.1 $[M]^+$.